

PL 1034124

REC'D 10 JUL 2003

WIPO PCT

THE UNITED STATES OF AMERICA

TO ALL TO WHOM THESE PRESENTS SHALL COME:

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office

July 03, 2003

THIS IS TO CERTIFY THAT ANNEXED HERETO IS A TRUE COPY FROM THE RECORDS OF THE UNITED STATES PATENT AND TRADEMARK OFFICE OF THOSE PAPERS OF THE BELOW IDENTIFIED PATENT APPLICATION THAT MET THE REQUIREMENTS TO BE GRANTED A FILING DATE.

APPLICATION NUMBER: 60/385,232

FILING DATE: June 03, 2002

RELATED PCT APPLICATION NUMBER: PCT/US03/17540

By Authority of the
COMMISSIONER OF PATENTS AND TRADEMARKS



P. SWAIN
Certifying Officer

**PRIORITY
DOCUMENT**

SUBMITTED OR TRANSMITTED IN
COMPLIANCE WITH RULE 17.1(a) OR (b)

BEST AVAILABLE COPY

1036 U.S. PTO
60/365232
06/03/02

Atty. Dkt. No. 082734-0102

1036 U.S. PTO
60/365232
06/03/02

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant: Robert E. CLICK
Title: NOVEL BACTERIUM FOR TREATMENT OF DISEASE
Appl. No.: Unknown
Filing Date: Submitted Herewith
Examiner: Unknown
Art Unit: Unknown

PROVISIONAL PATENT APPLICATION
TRANSMITTAL

BOX PROVISIONAL PATENT APPLICATION
Commissioner for Patents
Washington, D.C. 20231

Sir:

Transmitted herewith for filing under 37 C.F.R. § 1.53(c) is the provisional patent application of:

Robert E. CLICK

☒ Applicant claims small entity status under 37 CFR 1.27(c)(1).

Enclosed are:

- ☒ Specification, Claim(s), and Abstract (21 pages).
- ☐ Informal drawings (??? sheets, Figures 1-???).
- ☒ Application Data Sheet (37 CFR 1.76).

The filing fee is calculated below:

	Rate	Fee Totals
Basic Fee	\$160.00	\$160.00
[X] Small Entity Fees Apply (subtract ½ of above):	=	\$80.00
TOTAL FILING FEE:	=	\$80.00

- [X] A check in the amount of \$80.00 to cover the filing fee is enclosed.
- [] The required filing fees are not enclosed but will be submitted in response to the Notice to File Missing Parts of Application.
- [] Further, Applicant, by and through his attorney of record, hereby expressly abandons the application as of the filing date of this letter. This is an abandonment of the application only, and is not to be construed as an abandonment of the invention disclosed in the application. It is respectfully requested that the Office acknowledge abandonment of the application as of the filing date of this letter in a communication mailed to the undersigned.
- [X] The Commissioner is hereby authorized to charge any additional fees which may be required regarding this application under 37 C.F.R. §§ 1.16-1.17, or credit any overpayment, to Deposit Account No. 06-1447. Should no proper payment be enclosed herewith, as by a check being in the wrong amount, unsigned, post-dated, otherwise improper or informal or even entirely missing, the Assistant Commissioner is authorized to charge the unpaid amount to Deposit Account No. 06-1447.

Please direct all correspondence to the undersigned attorney or agent at the address indicated below.

Respectfully submitted,

Date June 3, 2002

By 

FOLEY & LARDNER

Customer Number: 23524



23524

PATENT TRADEMARK OFFICE

Telephone: (608) 258-4272

Facsimile: (608) 258-4258

Mark A. Kassel
Attorney for Applicant
Registration No. 38,200



UP UT ..

A/pn

PATENT APPLICATION

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants: Robert E. CLICK

Title: NOVEL BACTERIUM FOR TREATMENT OF DISEASE

Docket No.: 082734-0102

BOX PROVISIONAL PATENT APPLICATION
Commissioner for Patents
Washington, D.C. 20231

"Express Mail" Mailing Label No.: EL846155990US
Date of Deposit: June 3, 2002

I hereby certify that these attached documents

- * Transmittal of Provisional Patent Application (duplicate)
- * Provisional Patent Application: Specification (17 pages), Claims (3 pages) and Abstract (1 page)
- * Check (No. 32987) for \$80.00

are being deposited with the United States Postal Service "Express Mail Post Office to Addressee" service under 35 C.F.R §1.10 on the date indicated above and is addressed to BOX PROVISIONAL PATENT APPLICATION, Commissioner for Patents, Washington, D.C. 20231.

Enclosed for filing please find the above-referenced provisional patent application. Please indicate receipt of this provisional patent application by returning the attached postcard with the official Patent and Trademark Office receipt stamped thereon.

Cindy Blazek
Cindy Blazek
Foley & Lardner
P. O. Box 1497
Madison, Wisconsin 53701-1497
(608) 257-5035

Application Data Sheet

Application Information

Application Type:: Provisional
Subject Matter:: Utility
Suggested classification::
Suggested Group Art Unit::
CD-ROM or CD-R?:: None
Computer Readable Form (CRF)?:: No
Title:: NOVEL BACTERIUM FOR TREATMENT OF
DISEASE
Attorney Docket Number:: 082734-0102
Request for Early Publication?:: No
Request for Non-Publication?:: No
Suggested Drawing Figure::
Total Drawing Sheets::
Small Entity?:: Yes
Petition included?:: No
Secrecy Order in Parent Appl.?:: No

Applicant Information

Applicant Authority Type:: Inventor
Primary Citizenship Country:: US
Status:: Full Capacity
Given Name:: Robert E.
Family Name:: CLICK
City of Residence::

Country of Residence::

Street of mailing address::

Correspondence Information

Correspondence Customer Number:: 23524

E-Mail address:: mkassel@foleylaw.com

Representative Information

Representative Customer Number::	23524	
---	-------	--

Domestic Priority Information

Application::	Continuity Type::	Parent Application::	Parent Filing Date::

Foreign Priority Information

Country::	Application number::	Filing Date::	Priority Claimed::

Assignee Information

Assignee name::

60385232.060302

NOVEL BACTERIUM FOR TREATMENT OF DISEASE

TECHNICAL FIELD

[0001] The present invention relates to a novel bacterium and the use of the novel bacterium and other members of its genus, active portions thereof and/or proteins therefrom to treat a variety of diseases. More specifically, the invention relates to the use of a bacterium species within the genus *Dietzia*, including the bacterium *Dietzia* sp. C79793-74, and other bacteria isolated by an *in vitro* inhibition procedure used to isolate *Dietzia*, portions and/or proteins produced therefrom, to treat paratuberculosis of ruminants and mycobacterial-caused diseases of humans, such as Crohn's disease, leprosy, Sarcoidosis, and tuberculosis (TB).

BACKGROUND OF THE INVENTION

[0002] Paratuberculosis, or Johne's disease, in ruminants is a chronic infection of the gastrointestinal tract. *Mycobacterium paratuberculosis* (*MpT*) is known to be the causative agent of Johne's disease. Most animals that contract Johne's disease become infected shortly after birth and do not become clinically ill until they are adults. Infection is permanent, there are no viable treatments, and, if not culled, most clinically sick animals eventually die of wasting.

[0003] Current estimates indicate that 3.4% of dairy cows in 21.6% of herds and 0.9% of beef cattle in 7.9% of herds in the United States are infected with *MpT*. Similar data are reported in essentially all other countries. These results demonstrate that Johne's disease is a very serious worldwide problem. Indeed, it has been estimated to have a \$0.2 - 1.5 billion dollar economic impact in the United States due to loss of milk income, increased culling, low culling weights, extended calving intervals, and the unmarketability of breeding stock. Similarly, large economic losses from Johne's disease are also suffered in the sheep industry.

[0004] It is generally accepted that for clinical manifestation of paratuberculosis to occur, infection with *MpT* must occur at a young age. To date, no effective treatment has been reported for infected animals. Moreover, no prophylaxis has been reported that prevents infection. Biosecurity and other management practices have been proposed as a means to control the spread of Johne's disease. It is agreed that the spread of *MpT* could be lessened by: a) persistent attention to detail; b) avoidance of contact with fecal material from infected animals; and c) culling all infected and all offspring born to infected cattle. However, from a practical perspective, it has been suggested that management alone will likely fail to control infections. Thus, there is a need for viable methods to prevent and control paratuberculosis.

[0005] In addition to animals, mycobacteria are also major pathogens of humans. There are approximately ten million cases of tuberculosis worldwide with an annual mortality of three million. Leprosy, caused by *Mycobacterium leprae*, afflicts over ten million people, primarily in developing countries. *Mycobacterium tuberculosis* and mycobacteria of the *Mycobacterium avium-intracellulare* group are major opportunistic pathogens of immuno-compromised patients such as AIDS patients. Crohn's disease and Sarcoidosis are postulated to be a result of *MpT* infection. Most treatments for these diseases require intense and lengthy combinational drug therapy. Such treatments not only allow resistant strains to arise, but for Crohn's patients lasting resolution of disease has yet to be established. Thus, there is a need for more effective treatments for mycobacterial-incited diseases of humans.

[0006] Crohn's disease is a chronic, debilitating and potentially fatal disease that bears extensive clinical, pathologic, and systemic similarity to Johne's disease. In the United States, the number of newly diagnosed Crohn's patients is estimated to be 20,000 each year. Crohn's disease is a *granulomatous ileo-colitis* of unknown etiology. Postulates on the immunopathogenesis of Crohn's disease are that the disease results from an antigenic challenge to the gut-associated lymphoid tissues (GALT). Once triggered, cytokines and other inflammatory mediators released result in chronic and persistent inflammation. This inflammatory reaction is postulated to be the result of hyper-responsiveness of GALT to antigens present within intestinal cells. This hyper-reactivity may be a result of an

immunoregulatory defect or from a persistent stimulus, such as *MpT* antigens. Recent evidence defining a genetic predisposition is consistent with this theory. Thus, there is a need for treatments for Crohn's disease that target *MpT* in particular.

[0007] Animals afflicted with Johne's disease may be the source of *MpT* that underlie Crohn's disease in humans. For example, *MpT* may be transmitted to humans through contaminated meat and/or pasteurized milk. *MpT* is an intracellular pathogen that colonizes and multiplies in phagocytic cells present in blood and other tissues. Since phagocytes are natural constituents of milk, it is not surprising that *MpT* is found in milk of infected cows. Moreover, *MpT* is partially resistant to pasteurization presently used commercially.

Furthermore, animals that are heavily infected with *MpT* are usually culled and likely used in the production of ground beef. Inadequate cooking would again result in live *MpT* being present in food. Thus, any effective treatment of paratuberculosis is likely to reduce the transmission of *MpT* to humans, which could in turn result in a lower incidence of Crohn's disease.

SUMMARY OF THE INVENTION

[0008] The present invention relates to the use of bacteria of the genus *Dietzia* for the treatment of various diseases. The invention includes the use of an isolated bacterium identified as the *Dietzia sp. C79793-74* and deposited with the American Type Culture Collection (ATCC) as Accession Number PTA-4125, for the treatment of various diseases.

[0009] In one embodiment, the invention includes a composition suitable for administration to an animal comprising a carrier and one or more bacteria, the bacteria species selected from the genus *Dietzia*, including ATCC Accession Number PTA-4125, referred to collectively herein as "The Bacteria".

[0010] The invention further includes a composition suitable for administration to an animal comprising a carrier and a pharmacologically active, or recombinant DNA-derived,

dose of The Bacteria, active fragments of The Bacteria, including active fragments of enzymes, active molecules secreted from The Bacteria or from organisms with The Bacteria-derived DNA, and combinations of the above. Active molecules include proteins, DNA molecules, RNA molecules, carbohydrates, and components of the cell envelope, such as lipoglycans.

[0011] In another embodiment, the invention includes compositions that are suitable for administering The Bacteria to animals affected by Johne's disease. For example, the invention encompasses feed compositions and feed additives in which The Bacteria is an ingredient.

[0012] In still another embodiment, the invention includes a composition suitable for administration to a human comprising a suitable carrier and The Bacteria.

[0013] The invention further includes a composition suitable for administration to a human comprising a carrier and a pharmacologically active, or recombinant DNA-derived, dose of The Bacteria, active fragments of The Bacteria, active molecules secreted from The Bacteria or from organisms with The Bacteria-derived DNA, and combinations of the above. Methods for producing bacteria through recombinant processes are well known in the art.

[0014] In addition, the invention relates to methods of treating Johne's disease, Crohn's disease, and other mycobacterial-caused diseases by administering a composition comprising The Bacteria. In one embodiment, the invention includes a method of treating Johne's disease comprising orally administering a composition comprising The Bacteria to any mammals infected with *MpT*.

[0015] The bacteria used in the present invention are isolated from an animal sample, which may be the feces of bovine, co-culturing the bacterium with *MpT*, and selecting bacteria that inhibit the growth of *MpT*. In other embodiments, the bacterium are (or bacteria is) isolated from soil, sea water, or even insects.

[0016] The method also relates to a method of culturing bacteria of the genus *Dietzia* in a medium containing Tryptose soy broth.

60365232-050302

[0017] Further objects, features and advantages of the invention will be apparent from the following detailed description when taken in conjunction with the accompanying drawings.

DETAILED DESCRIPTION OF THE INVENTION

[0018] All ranges recited herein include all combinations and sub-combinations included with that range limits; therefore, a range from about "15 to about 60" would include ranges from about 15 to about 45, from about 30 to about 47, etc. A range of "up to 85" would include up to 80, up to 50, up to 24, etc.

[0019] The present invention relates to novel bacteria such as those species within the *Dietzia* genus ("The Bacteria"), that, when co-cultured with a pathogenic mycobacterium, inhibit the growth of the co-cultured mycobacterium and therefore prove useful in controlling the growth of said mycobacterium.

[0020] The Bacteria have also surprisingly and unexpectedly proved to be effective in treating *MpT* infected cattle, including those with and those without clinical disease.

[0021] Specifically, administering The Bacteria to ruminants has solved a longstanding problem in that it has proved to be effective for treating *MpT*-infected ruminants. It has been surprisingly and unexpectedly discovered that the oral administration of The Bacteria in a pharmacologically active dose to cattle treats the symptoms of Johne's disease and results in long term health benefits to the administered ruminant.

[0022] The bacterium is effective to treat Johne's disease in any ruminant, including, but not limited to, bovine, ovine, caprine, and cervine. Administration of The Bacteria to clinically, asymptomatic *MpT*-infected ruminants is also useful in preventing the onset of clinical symptoms of Johne's disease. In one embodiment, the ruminants include bovines, such as dairy calves and adult lactating and nonlactating cows. In another embodiment, the ruminants include caprine or ovine.

[0023] The Bacteria in one embodiment is administered to the ruminant orally. The Bacteria may be administered in a solution, mixed with a feed and/or mixed with a feed additive. The oral administration is given in a series of doses administered daily for a period of 3 - 10 months. When the ruminant is a bovine, the treatment is preferably administered for at least 6 months at a dosage of at least 10¹¹ cfu. In one embodiment, The Bacteria are administered orally to adult clinically-ill or asymptomatic dairy cows on a daily basis for about 3 - 10 months. However, any acceptable time period may be used as long as a pharmacologically effective dose is administered such that paratuberculosis is effectively prevented and/or cured. For example, if The Bacteria is mixed with a feed and/or feed additive, ruminants may be fed on a constant diet of feed containing a pharmacologically effective dose of The Bacteria until a total pharmacological dose results in cure and/or prevention of disease.

[0024] In one embodiment, 2 x 10¹¹ cfu of The Bacteria are administered daily, in a single dose. If The Bacteria are incorporated into feed, a range of concentrations of The Bacteria in the feed are appropriate to practice the method of the invention, in one embodiment about 10¹¹ - 10¹³ cfu per day. In one embodiment, The Bacteria are incorporated into the feed at a concentration such that 2 x 10¹¹ cfu of The Bacteria are administered in an amount of feed that is consumed in a single day. Any appropriate amount of The Bacteria, or active portions or proteins thereof, may be incorporated into the feed as long as a pharmacologically active dose of the material is fed to the animal within a daily feed. If The Bacteria are incorporated into a feed additive, a range of concentrations of The Bacteria in the feed additive are appropriate to practice the method of the invention, in one embodiment about 10¹¹ - cfu per day. In one embodiment, The Bacteria are incorporated into the feed additive at a concentration such that 2 x 10¹¹ cfu of The Bacteria are administered in an amount of feed that is consumed in a single day.

[0025] It is not intended that the present invention be limited by the particular nature of the therapeutic preparation. The Bacteria can be prepared in any type of appropriate medium for administration. For example, The Bacteria can be provided together with physiologically

tolerable liquid (e.g., saline), gel or solid carriers, diluents, adjuvant, excipients and as a rumen-protected encapsulated ingredient. Suitable diluents and excipients include pharmaceutical grades of physiological saline, dextrose, glycerol, mannitol, lactose, starch, magnesium stearate, sodium saccharin, cellulose, magnesium carbonate, and the like, and combinations thereof. In addition, if desired the compositions may contain minor amounts of auxiliary substances such as wetting or emulsifying agents, stabilizing or pH buffering agents. These compositions typically contain 1% - 95% of active ingredient, preferably 2% - 70%. The Bacteria can be incorporated into tablets, boluses, or capsules, and dosed to the patient. The Bacteria may also be incorporated into salt blocks and the like. The Bacteria can be added to feed as a freeze-dried powder or as an encapsulated bacteria that is protected from degradation in the rumen. In one embodiment, The Bacteria is prepared in a solution of physiological phosphate-buffered saline or in the culture medium, Tryptose soybean broth (TSB) for oral administration to a ruminant.

[0026] The Bacteria can be incorporated into tablets, drenches, boluses, capsules or premixes. Formulation of these active ingredients into such dosage forms can be accomplished by means of methods well known in the pharmaceutical formulation arts. See, for example, U.S. Patent Number 4,394,377. (This patent, and all other references cited herein are hereby incorporated by reference.) Filling gelatin capsules with any desired form of the active ingredients readily produces capsules. If desired these materials can be diluted with an inert powdered diluent, such as sugar, starch, powdered milk, purified crystalline cellulose, or the like to increase the volume for convenience of filling capsules.

[0027] Conventional formulation processes can be used to prepare tablets containing The Bacteria. In addition to the active ingredients, tablets may contain a base, a disintegrator, an absorbent, a binder, and a lubricant. Typical bases include lactose, sugar, sodium chloride, starch and mannitol. Starch is also a good disintegrator as is alginic acid. Surface-active agents such as sodium lauryl sulfate and dioctyl sodium sulphosuccinate are also sometimes used. Commonly used absorbents include starch and lactose. Magnesium carbonate is also useful for oily substances. As a binder there may be used, for example, gelatin, gums, starch,

00334558.6

dextrin, polyvinyl pyrrolidone and various cellulose derivatives. Among the commonly used lubricants are magnesium stearate, talc, paraffin wax, various metallic soaps, and polyethylene glycol.

[0028] Drenches are prepared most readily by choosing a saline-suspended form of The Bacteria, fragments thereof or active molecules secreted therefrom. A water-soluble form of one ingredient may be used in conjunction with a water-insoluble form of the other by preparing a suspension of one with an aqueous solution of the other. Water-insoluble forms of either active ingredient may be prepared as a suspension or in some physiologically acceptable solvent such as polyethylene glycol. Suspensions of water-insoluble forms of either active ingredient can be prepared in oils such as peanut, corn, sesame oil or the like; in a glycol such as propylene glycol or a polyethylene glycol; or in water depending on the solubility of a particular active ingredient. Suitable physiologically acceptable adjuvants may be necessary in order to keep the active ingredients suspended. The adjuvants can be chosen from among the thickeners, such as carboxymethylcellulose, polyvinyl pyrrolidone, gelatin and the alginates. Surfactants generally will serve to suspend the active ingredients, particularly the fat-soluble propionate-enhancing compounds. Most useful for making suspensions in liquid nonsolvents are alkylphenol polyethylene oxide adducts, naphthalenesulfonates, alkylbenzene-sulfonates, and the polyoxyethylene sorbitan esters. In addition many substances, which affect the hydrophilicity, density and surface tension of the liquid, can assist in making suspensions in individual cases. For example, silicone anti-foams, glycols, sorbitol, and sugars can be useful suspending agents.

[0029] Additionally the subject compositions of this invention may be separately administered, for example, by adding one directly to feed stuffs and co-administering the second material as a bolus tablet, drench, or capsule. Or each may be separately prepared and separately added to feed stuffs in appropriate quantities and at appropriate times. For example, such a material as choline stearate, a fatty acid complex which may be used in the practice of this invention, may not be appropriate for incorporation into feed premixes because of its physical characteristics. In such an instance the choline stearate composition could be provided

separately in a suitable diluent such as, for example, corn flour, ground corn cob, hominy, corn glutenmeal, wheat middlings, soybean meal, soybean mill feed, rice mill by-product, and the like and mixtures thereof. A description of such suitable diluents may be found in U.S. Patent Number 4,394,377.

[0030] The Bacteria may be administered to an animal in a composition, a premix, that is then mixed into the animal feed supply. Such a composition may comprise The Bacteria alone or The Bacteria may be mixed with a carrier and/or with other drugs, vitamins, minerals, protein concentrates and similar feed supplements. These compositions may be prepared in dry granular powder form, as pellets, in the form of pastes, encapsulated to be rumen protected, or may be formulated as liquid feed supplements and the like. Any type of feed may be medicated with such compositions, including common dry feed, liquid feeds, and pelleted feeds. The methods of formulating supplemental materials into animal feeds are well known. It is necessary only to calculate the amount of each compound, which it is desired to administer to each animal, to take into account the amount of feed per day that the animal eats and then mix in the appropriate amount of The Bacteria. See U.S. Patent Number 4,394,377.

[0031] The compositions of the invention may be used as a feed additive premix, feed additive concentrate or feed additive supplement in which the active ingredients are distributed uniformly throughout a standard organic or inorganic animal feed carrier in a concentrated form which is conveniently packaged and shipped to the feed mixer. The grower or the feed mixer then in turn mixes this premix, concentrate or supplement uniformly with a normal diet for the animal as desired. Examples of carriers for premix compositions are soybean meal, corn oil, ground corn, barley, wheat, mineral mixtures containing, e.g., vermiculite or diatomaceous earth, corn gluten meal, soy flour or other modestly priced edible ingredients.

[0032] The Bacteria may also be admixed with a suitable carrier such as an edible feed or feed component in the form of a feed additive supplement. Examples of such edible feed components are feed fortifiers and enhancers for preruminant bovine calves of any of the kinds disclosed in U.S. Patent Number 6,156,333. If to be fed free choice or as a supplement, The

Bacteria is provided according to the anticipated daily consumption of the supplement to provide a daily dose of each of these ingredients in one of the ranges specified.

[0033] In addition, The Bacteria may be incorporated directly into feeds by a mill or other feed supplier to provide a finished feed product to the grower. A finished feed product could be made up of any of the various grains, lucerne, grasses, minerals, vitamins, protein supplements, drugs and the like which go into the formulation of a nutritionally complete ruminant feed. The Bacteria may be mixed directly with cattle feed made up of various components such as hay, straw, silage, cornstalks, cottonseed hulls, grain, oats, barley and cereal brans, particularly for the ruminants; antioxidants, minerals, vitamins, anthelmintics, and other appropriate medicaments. See U.S. Patent Number 4,394,377. Alternatively, The Bacteria may be incorporated into a liquid feed for preruminant bovine calves of any of the kinds disclosed in U.S. Patent Number 6,156,333.

[0034] The Bacteria may be mixed into a suitable animal feed by any method appropriate for mixing a bacterium into animal feed. Examples of such methods include but are not limited to the following: spraying The Bacteria onto dry feed and mechanically mixing The Bacteria into dry or liquid feed; top dress grain or concentrate mix.

[0035] The Bacteria of the present invention are also useful in treating medical conditions in humans that result from various mycobacterial infections. These include Crohn's, leprosy, tuberculosis, Sarcoidosis, and diarrhea in immuno-compromised (AIDS) patents. Administration of The Bacteria or active fragments, proteins, secretions, etc. thereof by appropriate means known in the art to a human patient should demonstrate a reduction of the symptoms of the disease or syndrome caused by the mycobacterium.

[0036] *Dietzia sp.* 79793-74, ATCC Accession Number PTA-4125 was identified as a unique bacterial contaminant during culturing of feces from *MpT* sero-positive and negative cows. The bacterial contaminant was subsequently isolated, cultured, and identified as being of the *Dietzia* genus. On further investigation, *Dietzia sp.* 79793-74 was found to completely

inhibit the growth of *MpT* when co-cultured with *MpT*. This method of utilizing a sample isolated from an appropriate animal host may be used to isolate other bacteria, in addition to those of the *Dietzia* genus, that inhibit the growth of *MpT* and that are therefore useful for treating diseases caused by *MpT*.

[0037] Other micro-organisms that inhibit the growth of various mycobacteria may be isolated by similar means. Other mycobacteria that may be inhibited include *M. tuberculosis*, *M. leprae*, *M. avium-intracellulare*, *M. bovis*, *M. chelonae* (also known as borstelense and abscessus), *M. africanum*, *M. marinum* (also known as balnei and platypocillus, the causative agent of "swimming pool granuloma"), *M. buruli* (also known as ulcerans), *M. fortuitum* (also known as giae, minetti, and ranae), *M. haemophilum*, *M. intracellulare*, *M. kansasii* (also known as luciflavum), *M. littorale* (also known as xenopi), *M. malmoense*, *M. marianum* (also known as scrofulaceum and paraffinicum), *M. simiae*, *M. szulgai*, and *M. ulcerans* (which is the agent responsible for Buruli ulcer), *M. avium* (also known as brunense), *M. flavescens*, *M. lepraemurium*, and *M. microti*.

[0038] Bacteria of the *Dietzia* genus may be cultured by growing the bacteria on agar slants in Tryptose soy broth at 35°C. Large-scale production of *Dietzia* genus bacteria is achieved by growth in suspension in large biofermenters. Viability of such bacteria is unchanged by storing at -20°C. In addition, bacteria of the *Dietzia* genus can be stored as a freeze-dried material at room temperature. Other storage methods that maintain bacterial viability that are known in the art may be used.

[0039] Active fragments of bacteria from the *Dietzia* genus and active molecules isolated from such bacteria may be prepared by any known method for preparing/identifying active fragments of bacteria and proteins secreted from bacteria. Such methods include but are not limited to the following: sonication, osmotic shock, detergent lysis, high pressure, transfer appropriate DNA to other organisms, such as bacteria, plant or animal that is then used as a feed additive as described previously.

[0040] The compositions and methods of the invention create several important benefits: an effective treatment for Johne's disease is provided which will increase profitability for farmers; products such as milk and meat will be safer for human consumption by eliminating potential *MpT* contamination; and a potentially effective non-antibiotic treatment for Crohn's patients as well as other diseases caused by mycobacteria, such as tuberculosis and leprosy. The methodology also teaches a way to identify bacteria that inhibit the growth of various mycobacteria.

[0041] Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, numerous equivalents to the specific embodiments described herein. Such equivalents are within the scope of this invention.

EXAMPLES

[0042] The invention is further described in the following, non-limiting examples.

Example 1. Antibodies in sera and colostrum/milk from *Dietzia*-sensitized cows cross-react with *MpT* as determined in an ELISA assay

[0043] Healthy cows that tested negative for *MpT* infection were injected with the indicated doses of *Dietzia sp.* C79793-74, Accession No. PTA-4125. The titers of antibodies that are cross-reactive with *MpT* in milk and sera taken from the injected cows were determined by a standard ELISA assay that those skilled in the art will recognize or be able to ascertain.

Table 1

Cow ID #	Injection		ELISA Titers		
	Date	Dose	Date	Milk	Serum
51	None		7/21/94	-	ND
	2/03/95	2x10 ¹⁰ IM	2/03/95	ND	-
	2/17/95	6x10 ¹⁰ IM	2/17/95	ND	ND
	3/14/95	4x10 ⁹ IM	3/14/95	+	4.6
	4/04/95	4x10 ⁹ IM	5/09/95	++	ND
			7/28/95	+	ND
			3/08/96	ND	3.2
B-5	None		7/21/94	-	ND
	2/03/95	2x10 ¹⁰ IM	2/03/95	ND	-
			3/14/95	ND	2.3
	4/04/95	4x10 ⁹ IM	5/09/95	-	ND
			7/27/95	+	ND
			3/08/96	ND	-
B-10	None		7/21/94	-	ND
			2/03/95	ND	-
	2/17/95	4x10 ¹⁰ IM	3/14/95	ND	2.5
	3/14/95	4x10 ⁹ IM	5/09/95	-	ND
	4/04/95	4x10 ⁹ IM	3/08/96	ND	2.1

Key: - means negative; + means positive; ND means not done; IM means intramuscularly

[0044] The results in Table 1 show that cows immunized with *Dietzia* bacteria ATCC Accession No. PTA-4125 produce antibodies that cross-react with *MpT*. Moreover, these antibodies are found in both serum and milk. The results are important because they provide a possible explanation for the mechanism of action of the *Dietzia* bacteria: phagocytes infected with *MpT* can more readily take up *Dietzia* bacteria coated with antibodies that cross-react with *MpT*. Thus, the cross-reactive antibodies may serve to deliver *Dietzia* bacteria to cells infected with *MpT*.

Example 2. Treatment of cows that are sero-positive for Johne's disease

[0045] Cows afflicted with Johne's disease were treated according to the method of the invention by administration of *Dietzia* bacteria ATCC Accession No. PTA-4125. The clinical results with seven such cows are shown in Table 2 below. Each cow was administered a single oral dose of 2×10^{11} cfu, on a daily basis, in a single dose, of *Dietzia* sp. C79793-74, ATCC Accession No. PTA-4125.

Table 2

Cow ID #	Calving Dates	Treatment Length	Treatment
H-83	8/27/97 4/20/99 7/20/00 8/08/01	9 months	Began showing clinical symptoms on 1/1/98. Began daily treatments 2/25/98. Recovered on 4/1/98. Had a relapse on 10/18/98 and recovered by 10/23/98. Stopped treatment on 11/13/98. She has not had any further relapses but did have a borderline serum ELISA on 10/15/99 which returned to negative on 6/28/00 and 8/20/01. Fecal samples taken 11/29/99, 6/28/00, 1/1/01 and 8/20/01 were negative.
H-51	11/5/96 7/17/98	3 months	Signs of disease on 8/1/98. Started treatment on 8/31/98. Recovered. No further symptoms.
H-81	8/30/97 3/26/99	6 months	Broke on 5/24/98; started treatment on 5/25/98. Recovered. No further symptoms.
H-58	11/3/96	10 months	Signs of disease on 1/10/98. Started treatment on 1/25/98. Recovered. No further symptoms.
H-62	10/25/96 2/1/98	10 months	Signs of disease on 1/20/98. Started on treatment on 1/20/98. Recovered. Relapse on 10/29/98. Recovered. No further symptoms.
H-53	11/3/96 9/4/98	5 months	Signs of disease on 4/7/98; Started treatment same day. Recovered. Moved to maternity pen on 8/26/98. She quit eating on 9/1/98, calved with difficulty and died with no clinical signs.
H-64	10/21/96 3/26/98	7 months	Minor signs of disease in first lactation. Relapse during 2nd lactation on 4/7/98. Started treatment on 4/7/98. Although there were times when she appeared to recover, we were not able to turn her around. She eventually died on 11/5/98 with severe diarrhea and was quite emaciated.

[0046] The results in Table 2 show that treatment of *MpT* sero-positive animals with *Dietzia sp. C79793-74*, ATCC Accession No. PTA-4125 is effective (and for H-83, curative). For instance, cow H-83, who was treated with *Dietzia sp. C79793-74*, ATCC Accession No. PTA-4125 for 9 months remains in the herd as of 5/01/02, ELISA negative, culture negative and clinically free, 38 months after treatment was terminated. Results also suggest that the best candidates for treatment are those with early-stage disease. Any results such as those shown in Table 2, or similar thereto depending on the mammal to be treated is said to meet the definition of "reducing or preventing the symptoms of a disease or syndrome" as discussed in this application.

[0047] To summarize, a clinical study was undertaken in which adult dairy cattle that displayed symptoms of Johne's disease were administered *Dietzia sp. C79793-74*, ATCC Accession No. PTA-4125. In this study, the bacteria was given daily for 3 - 10 months. Of seven clinically sick animals treated, six recovered. Moreover, four treated animals that were *MpT*-sero-positive, but clinically asymptomatic, never developed disease. In contrast, the ten *MpT*-clinically sick animals that were not treated went on to succumb with overt clinical disease.

Example 3. Safety of BC: Administration of *Dietzia* to calves and mice

[0048] In addition to the cows that were administered live or killed *Dietzia sp. C79793-74*, ATCC Accession No. PTA-4125, a number of calves and mice were also administered the same live bacteria. Two types of mice were used. Eight immunologically competent, 2 - 3 month old A/J males and eight females (two of each per cage) were injected IP with 108 cfu live *Dietzia sp. C79793-74*, ATCC Accession No. PTA-4125. They were then monitored for ten months for any signs of disease (weight loss, diarrhea) and reproductive problems. All activities monitored were indistinguishable from those of untreated (control) animals. In addition, 108 cfu live *Dietzia sp. C79793-74*, ATCC Accession No. PTA-4125 were injected intraperitoneally (IP) into mouse CB.17 severe-combined-immunodeficient mice (SCID) mice. Just as was found with conventional mice, the injected bacteria manifested no

detrimental overt reaction even though these mice, genetically, are immunologically incompetent (they lack functional T and B cells).

[0049] Seven bull calves destined to become steers were used to determine the safety of 109 cfu *Dietzia* sp. C79793-74, ATCC Accession No. PTA-4125 given orally, as a single dose, on a daily basis, from birth up to 15 days. Two bull calves served as controls and did not receive any of the bacteria. All animals were castrated and raised for slaughter. During their lives of 15 - 18 months, no signs of disease or sickness were obvious. They were all similar in weight at slaughter. At approximately four months of age, a sample of blood was taken from all nine calves from which the prepared sera was tested for antibodies to *MpT* in an ELISA assay. All animals were found to be negative, except one calf that had a positive ELISA reading. This was expected since this calf received colostrum from a *Dietzia* sp. C79793-74 hyper-immunized cow (H-51 in Table 1) possessing antibodies in her milk.

[0050] The above results show that live *Dietzia* sp. C79793-74 are not pathogenic when administered orally or injected.

Example 4. Treatment of clinically sick and/or asymptomatic cows

[0051] A large study was initiated in which liquid or freeze-dried *Dietzia* sp. C79793-74 is given to clinically sick and/or asymptomatic cows that are either

- a) Sero-ELISA positive, Sero-AGID negative, fecal negative
- b) Sero-ELISA positive, Sero-AGID positive, fecal negative
- c) Sero-ELISA positive, Sero-AGID negative, fecal positive
- d) Sero-ELISA positive, Sero-AGID positive, fecal positive
- e) Sero-ELISA negative, Sero-AGID negative, fecal positive.

[0052] After 7 months, the preliminary results indicate that the dose of the bacteria necessary to prevent death of Johne's positive cows is dependent upon the body weight of the

animal being treated. Holsteins for example that weigh up to 2X more than Jerseys, need at least 2X higher dose of bacteria.

[0053] Animals that were Elisa positive and AGID positive were more difficult to maintain alive than those that were only Elisa positive. Survival did not appear to depend upon whether an animal shed or did not shed *MpT*.

[0054] Of 19 animals (out of 36) found to be shedding *MpT* in feces, after 6 weeks of treatment, only one was found to still be shedding. Of 8 animals that calved while on treatment, none had detectable *MpT* in their colostrums. This is contrary to what is published. These results suggest that it is possible that the treatment prevented shedding in colostrums.

[0055] A number of animals (12) died during treatment, most due to complications such as displaced abomasums. Only 4 died due to Johne's and they were all AGID positive when started on treatment.

[0056] In tissues of two animals that were euthanized and autopsied, no *Dietzia* were detected.

[0057] Weight changes post treatment of two animals that were or became clinically very sick are shown below. Both are still in the herd.

Cow #	weights on				
	9/1/01	10/24/01	12/06/01	1/2/02	2/10/02
G-4	945	1095	1160	1190	1090
R100	1000	840	940	1045	1030*

Weight prior to treatment * calved

[0058] Treatment appears to reduce shedding of *MpT* in both feces and milk. Very sick animals gain weight. No *Dietzia* was found systemically in autopsied cows.

CLAIMS

What is claimed is:

- 1 1. A composition comprising an isolated bacterium belonging to the genus
2 *Dietzia* and deposited with the American Type Culture Collection (ATCC) as Accession
3 Number PTA-4125.
- 1 2. A feed composition, comprising the bacterium of claim 1 and a suitable
2 animal feed.
- 3 3. A feed additive, comprising the bacterium of claim 1 and an ingredient
suitable for use in a feed additive.
- 4 4. A composition, comprising a pharmacologically active dose of the
bacterium of claim 1, an active fragment thereof, a protein secreted therefrom, or
combinations thereof.
- 5 5. A method of treating Johne's disease, comprising administering to a
mammal the composition of claim 4.
- 1 6. The method of claim 5, wherein the mammal is a bovine.
- 1 7. The method of claim 6, wherein the mammal is an ovine.
- 1 8. The method of claim 5, wherein the method comprises oral
2 administration of the composition.
- 1 9. A method of treating Crohn's disease, comprising administering to a
2 human the composition of claim 4.

1 10. A method of treating diarrhea, comprising administering to a human the
2 composition of claim 4.

1 11. A method for treating leprosy, comprising administering to a human the
2 composition of claim 4.

1 12. A method for treating tuberculosis comprising administering to a human
2 the composition of claim 4.

1 13. A method for treating Sarcoidosis comprising administering to a human
2 the composition of claim 4.

14. A method of culturing bacteria belonging to the genus *Dietzia* comprising
culturing the bacteria in a medium comprising Tryptose soy broth.

15. A feed composition, comprising a bacterium of the genus *Dietzia* and a
suitable animal feed.

16. A feed additive, comprising a bacterium of the genus *Dietzia* and an
ingredient suitable for use in a feed additive.

17. A composition, comprising a pharmacologically active dose of a
2 bacterium of the genus *Dietzia*, an active fragment thereof, a protein secreted therefrom, or
3 combinations thereof, such that the pharmacologically active dose is capable of reducing or
4 preventing the symptoms of a disease or syndrome whose causative agent is a mycobacterium.

1 18. A method of treating Johne's disease, comprising administering to a
2 mammal the composition of claim 17.

1 19. The method of claim 18, wherein the mammal is a bovine.

1 20. The method of claim 18, wherein the mammal is a ovine.

1 21. The method of claim 18, wherein the method comprises oral
2 administration of the composition.

1 22. A method of treating Crohn's disease, comprising administering to a
2 human the composition of claim 17.

20250901 2253209

NOVEL BACTERIUM FOR TREATMENT OF DISEASE

ABSTRACT OF THE DISCLOSURE

[0059] This invention relates to compositions comprising a bacterium of the genus *Dietzia* that is useful for treating paratuberculosis in ruminants and mycobacterial-incited diseases of humans, such as Crohn's. The invention further relates to methods of treating Johne's and Crohn's diseases by administering to a mammal a composition of the invention. The invention also comprises a method of culturing the bacterium.

6038533-060303

**This Page is Inserted by IFW Indexing and Scanning
Operations and is not part of the Official Record**

BEST AVAILABLE IMAGES

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images include but are not limited to the items checked:

- ☐ BLACK BORDERS
- ☐ IMAGE CUT OFF AT TOP, BOTTOM OR SIDES
- ☐ FADED TEXT OR DRAWING
- ☐ BLURRED OR ILLEGIBLE TEXT OR DRAWING
- ☐ SKEWED/SLANTED IMAGES
- ☐ COLOR OR BLACK AND WHITE PHOTOGRAPHS
- ☐ GRAY SCALE DOCUMENTS
- ☐ LINES OR MARKS ON ORIGINAL DOCUMENT
- ☒ REFERENCE(S) OR EXHIBIT(S) SUBMITTED ARE POOR QUALITY
- ☐ OTHER: _____

IMAGES ARE BEST AVAILABLE COPY.

As rescanning these documents will not correct the image problems checked, please do not report these problems to the IFW Image Problem Mailbox.